

§Appl. No. 09/646,924
Amdt. dated April 16, 2004
Reply to Office Action of, January 27, 2004

Listing of Claims:

Please **amend** the claims as follows:

Claim 1 (Currently Amended) A method of screening a substance for usefulness in the treatment of a lipid metabolism dysfunction comprising contacting said substance with a ROR α receptor, or ~~the~~ a response element of ROR α located at position -198 to +24 of the apo C-III promoter involved in the regulation of the apo C-III gene, and measuring the level of apo C-III gene expression.

Claim 2 (Cancelled)

Claim 3 (Currently Amended) A method of screening a substance for usefulness in the treatment of a lipid metabolism dysfunction, comprising contacting said substance with (a) a ROR α receptor involved in the regulation of the expression of the apo C-III gene, (b) ~~the~~ a response element of ROR α located at position -198 to +24 of the apo C-III promoter, or (c) a nuclear factor which functionally couples ROR α to a RNA polymerase complex, and then measuring:

- i) the binding of said substance to the ROR α receptor or the binding of the complex formed by said substance and the ROR α receptor to ~~its~~ the response element or to a nuclear factor which couples ROR α to a RNA polymerase complex;
- or
- ii) the modulation of the transcriptional activity of a gene placed under the control of a promoter comprising said response element.

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Claim 4 (Currently Amended) The method of screening according to claim 3, comprising:

- a) transfecting a cellular host with a DNA fragment encoding an ROR α receptor;
- b) cotransfecting the host in a) with a construct comprising a ~~the~~ response element and at least one reporter gene; and
- c) measuring the expression of the reporter gene in the presence of the test substance.

Claim 5 (Currently Amended) The method of screening according to claim 3, comprising:

- a) creating a plasmid which comprises several copies of ~~the~~ a response element recognized by ROR α cloned upstream of a strong heterologous promoter which controls the expression of a reporter gene;
- b) transfecting the construct of a) into host cells which express ROR α naturally or artificially;
- c) incubating the host cells of b) in the presence of the test substance; and
- d) measuring the activity of the reporter gene.

Claim 6 (Currently Amended) The method of screening according to claim 3, comprising:

- a) creating a plasmid which comprises several copies of a ~~the~~ response element recognized by ROR α cloned upstream of a promoter which controls the expression of a selectable gene;
- b) transfecting the construct of a) into a cellular host;
- c) cotransfecting the host of b) with the aid of a vector expressing ROR α ;
- d) incubating the host of c) in the presence of the test substance; and
- e) measuring the cellular survival of said cellular host in the presence of a toxic prodrug.

Claim 7 (Previously Presented) The method of screening according to claim 3, comprising:

- a) creating a plasmid which comprises several copies of a response element recognized by a yeast nuclear factor Gal4 cloned upstream of a strong promoter which controls the activity of a reporter gene;
- b) creating a plasmid from a chimera which comprises a DNA binding domain of Gal4 and a DEF domain of ROR α which are the ROR α domains to which the ligands bind;
- c) cotransfecting the plasmids in a) or b) into a cellular host;
- d) incubating the host of c) in the presence of a test substance; and
- e) measuring the activity of said reporter gene.

Claim 8 (Currently Amended) The method of screening according to claim 3, comprising:

- a) transforming the cellular host with a construct carrying a gene encoding a ROR α receptor or a the response element of a ROR α receptor, and;
- b) assaying said cellular host or an extract thereof for the competitive displacement in the binding of labeled and unlabeled ligand to said ROR α receptor.

Claim 9 (Currently Amended) The method of screening according to claim 4, wherein the construct carrying the gene encoding a ROR α receptor or a the response element of the ROR α receptor also comprises a reporter gene.

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Claim 10 (Previously Presented) The method of screening according to claim 9, wherein the reporter gene is chosen from chloramphenicol acetyltransferase, the gene for luciferase from firefly or from Renilla, the gene for secreted alkaline phosphatase, the gene for beta-galactosidase or the gene for apo C-III.

Claim 11 (Previously Presented) The method of screening according to claim 4, wherein the cellular host is chosen from mammalian cells, bacteria, yeasts, or insect cells.

Claim 12 (Previously Presented) The method of screening according to claim 3, wherein the effect of said substance on the expression of said apo C-III gene is determined using transfection or analysis of mRNAs *in vitro* or on models *in vitro* or *in vivo*.

Claim 13 (Cancelled)

Claim 14 (Previously Presented) A method for preparing a pharmaceutical composition or a medicament useful in treating or preventing atherosclerosis in humans or animals comprising selecting a substance screened according to claim 3.

Claim 15 (Previously Presented) A method for treating or preventing atherosclerosis in humans or animals comprising modulating the expression of apo C-III using a medicament or a pharmaceutical composition comprising a substance selected according to claim 3.

Claim 16 (Currently Amended) A method for treating or preventing atherosclerosis in humans or animals comprising administering a medicament or a pharmaceutical composition comprising a substance which binds to a ROR α receptor, or the a response element of ROR α located at position -198 to +24 of the apo C-III promoter involved in the regulation of the apo C-III gene.

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Claim 17 (Previously Presented) The method according to claim 3, wherein the substance has antiatherosclerotic properties.

Claim 18 (Previously Presented) A method of screening according to claim 8, wherein the construct carrying a gene encoding the ROR receptor or the response element of the ROR receptor also comprises a reporter gene.

Claim 19 (Previously Presented) The method according to claim 1, wherein the lipid metabolism dysfunction is atherosclerosis.

Claim 20 (Previously Presented) The method according to claim 2, wherein the lipid metabolism dysfunction is atherosclerosis.

Claim 21 (Previously Presented) The method of screening according to claim 4, wherein the lipid metabolism dysfunction is atherosclerosis.

Claim 22 (Previously Presented) A method of measuring the expression of the apo C-III gene, comprising contacting a substance with a ROR α receptor or a response element of the ROR α receptor located at position -198 to +24 of the apo C-III promoter or a nuclear factor which couples ROR α to a RNA polymerase complex, and then measuring:

i) the binding of said substance to the ROR α receptor or the binding of the complex formed by the said substance and the ROR α receptor to the response element or to a nuclear factor which couples ROR α to a RNA polymerase complex;

or

ii) the modulation of the transcriptional activity of a gene placed under the control of a promoter comprising said response element.

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Claim 23 (New) The method of screening according to claim 3, comprising:

- a) transfecting a construct into host cells which express ROR α naturally or artificially, wherein said construct comprises at least two copies of said response element cloned upstream of a heterologous promoter which controls the expression of a reporter gene,
- b) incubating the host cells of a) in the presence of the test substance; and
- c) measuring the activity of the reporter gene.

Claim 24 (New) The method of screening according to claim 3, comprising:

- a) transfecting a construct into host cells which express ROR α naturally or artificially, wherein said construct comprises at least two copies of said response element cloned upstream of a heterologous promoter which controls the expression of a selectable gene,
- b) incubating the host cells of a) in the presence of the test substance; and
- c) contacting cells with a toxic prodrug which is toxic to cells in the absence of the selectable gene, and
- d) measuring the survival of said host cells.

Claim 25 (New) A method of screening a substance for usefulness in the treatment of a lipid metabolism dysfunction comprising,

- contacting said substance with a ROR α receptor, and a response element of ROR α located at position -198 to +24 of the apo C-III promoter involved in the regulation of the apo C-III gene, and
- and measuring the binding of said receptor to said response element.

Claim 26 (New) A method of claim 25, wherein the measuring is performed by the gel retardation method.

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- Claim 27** (New) A method of claim 1, wherein said response element is from -108 to +24.
- Claim 28** (New) A method of claim 16, wherein said response element is from -108 to +24.
- Claim 29** (New) A method of claim 22, wherein said response element is from -108 to +24.
- Claim 30** (New) A method of claim 25, wherein said response element is from -108 to +24.